Correlating Obstructed Diffusion with Obstacle Morphology using Single Molecule Tracking and AFM in Supported Lipid Bilayers

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Biophysical research has shown that membrane phospholipids and proteins diffuse not only under normal Brownian diffusion, but with confined and abnormal behavior. We are motivated to understand this anomalous diffusion because it may be involved in many cell functions. We have used single molecule tracking on phase-separated, supported lipid bilayers to investigate the origin of this unusual diffusion. DSPC forms ~250 nm, gel phase domains that act as obstacles to diffusion in the DOPC continuous liquid phase. Incorporated into the fluid phase at very low concentration is DMPE labeled with Alexa Fluor 647. When combined with the low background of a single supported bilayer on a quartz substrate, this high quantum yield dye yields a signal to noise ratio greater than 10. By controlling the gel domain morphology and characterizing it with atomic force microscopy, we can correlate the observed single molecule diffusion with the obstacle characteristics. We also compare our results to simulated Brownian diffusion in the presence of experimentally determined obstacle fields. An understanding of this correlation will aid studies that cannot directly characterize the obstacles to diffusion.

Novel Probes for Sensing Lateral Stress in Membranes


An understanding of the link between lipid composition and biomechanical properties of the bilayer (e.g. the lateral pressure and bending rigidity) is key to understanding the mechanisms that underpin lipid-protein interactions. Whilst it is now possible to determine parameters such as the spontaneous curvature and bending rigidity of lipid bilayers, evaluation of the lateral pressures within membrane systems remains elusive. Here, we present a novel platform based upon fluorescent probes that are able to sense the stored stresses within lipid bilayers and show how these correlate with the make-up of the membrane. In particular, we have used this system to study the effect of phosphatidylinoirolipids upon model membrane systems.

Lipid Diffusion in Tethered Bilayer Lipid Membranes (tBLMs)

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The complexity of cells makes molecular-scale characterizations of structure and interactions of biomembranes in vivo extremely difficult, thus driving the development of synthetic membrane models. tBLMs are resilient biomimetic systems stabilized by the proximity of an inorganic interface. We characterize their in-plane structure, dynamics and dielectric properties using fluorescence microscopy, correlation spectroscopy (FCS) and electrochemical impedance spectroscopy (EIS). The in-plane dynamics of tBLMs depend on structural details of the anchor lipid and its lateral density in the bilayer leaflet proximal to the substrate. In tBLMs with homogeneous lateral label distributions, the fluidity of the distal leaflet is comparable to that in vesicle membranes (2D diffusion constant, D ~ 7 mm²/s) while that in the proximal leaflet is moderately reduced (D ~ 0.8 mm²/s) - at variance with some established models. We present results from QD-conjugated transferrin receptors in healthy and insulin-resistant adipocytes and the impact of chromium picolinate on receptor mobility.

Phenocid Phase Behavior and Molecular Interactions of Membranes Containing Phosphatidylcholines and Sterol: A Deuteration NMR Study

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We study the phase behavior and properties of model membranes containing DPPC and POPC with or without sterol using 2H-NMR. The sm-1 chains of POPC and DPPC are deuterium-labeled in turn, such that information regarding each lipid component can be obtained. NMR spectra were taken as a function of temperature. The chain order of DPPC is greater than that of POPC in all mixtures studied. In DPPC/POPC binary mixtures, coexistence of solid-ordered (so) and liquid-disordered (ld) phases is observed in a wide temperature range. The results for ternary mixtures show that the addition of sterol promotes the formation of liquid-ordered (lo) phase. Furthermore, the influence of ergosterol on the lipid-lipid interactions is not as robust as that of cholesterol. Cholesterol enhances the DPPC-POPC interaction significantly. The phase behaviors of ternary mixtures will be discussed.
peptide-lipid interactions. As opposed to most natural lipids, DPhPC is more chemically stable, and therefore, it can provide for a more robust model of lipid membranes. However, despite its frequent use, DPhPC has been less investigated than other PC lipids. To measure the physical properties of DPhPC membranes we employ a combination of complementary methods that include x-ray scattering, Nuclear Magnetic Resonance (NMR), and osmotic stress. By x-ray scattering, we obtain the repeat distances (D-spacings) of multilamellar structures formed by DPhPC in solution. By applying osmotic stress, we are able to modify and control intermembrane spacings and molecular conformations, the latter measured by NMR. These structural measurements together with ion channel activity measurements using the standard gramicidin A channels allow us to compare DPhPC with more common and better studied PC lipids. In this way, we can help rationalize the use of DPhPC as a model membrane for studies of membrane function.

3480-Pos
Protein-Lipid Interaction and Domain Formation in Asymmetric Membranes
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The lateral organization of lipids and receptors in cellular membranes is connected to a multitude of biological and pathological processes. The role of spin-golipid- and cholesterol-rich lipid “rafts” as membrane domains that control protein-protein interaction has recently attracted much attention. While the model membrane approach has yielded fundamental insights into cell membrane structure and function, the majority of these studies did not take into account the very important asymmetry between the composition of the inner and the outer leaflets of cell membranes. Therefore, certain key questions remain still unanswered: for example, how is the partition of membrane components in raft domains affected by the presence of non-raft lipids in the inner leaflet? To answer questions of this type we have developed a new method based upon cyclodextrin-induced lipid exchange to create giant unilamellar vesicles (GUVs) featuring the same type of asymmetry encountered in biological membranes. These vesicles can be produced with a very large yield, while avoiding use of organic solvents. The asymmetry of the bilayer can be confirmed by leaflet-targeted Fluorescence Correlation Spectroscopy (FCS). The use of asymmetric GUVs as novel model bilayers should allow the investigation of the principles underlying communication between raft-like domains in opposite leaflets of the bilayer, as well as investigation of the origin of lipid and membrane protein affinity for rafts.

3481-Pos
Thermal Energies and Invariant Lipid Structures
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Biological membranes pose many interesting problems amenable to a physicist’s perspective. A central effort of a physicist is to discover invariant properties as conditions and systems change. As an example, for a lipid bilayer system one can vary the type and length of the hydrocarbon chains, in addition to the specific headgroup. Furthermore, lipid bilayers in solution exhibit a range of molecular motions with amplitudes determined by the available thermal energy. How will membrane properties behave under these changes? The theoretical framework to answer this question is within classical equilibrium thermodynamics. We use a mean-torque potential model [1] to analyze order parameter data from solid-state NMR measurements on lipid bilayers to describe how bilayer properties scale with temperature and acyl chain length. We find an invariant description of acyl chain packing, which allows us to address the correspondence between changes in acyl length and changes in temperature. We present the functional form of this scaling relationship, the conclusions that can be drawn from such a temperature study, and how this invariance is ultimately a step in determining the guiding principles of lipid mixture organization in biological membranes.

3482-Pos
Specific Spatial and Orientational Order in Phospholipid Membranes Induced by Cholesterol
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Cholesterol plays a major role in formation of laterally ordered membrane structures such as lipid rafts. These domains have been found to be involved in a variety of cellular functions, implying that there is immediate interest to understand the structure as well as the dynamics of rafts, and in particular the role of cholesterol in promoting order in rafts. Nonetheless, due to the molecular scales associated with lipid rafts and the soft nature of membrane domains overall, the atomic-level mechanisms responsible for cholesterol’s specific ordering capability remain unresolved. Our atomistic simulations [1] reveal that this ordering and the associated packing effects in membranes largely result from cholesterol’s molecular structure, which differentiates cholesterol from other sterols. Cholesterol molecules are found to prefer a specific spatial and orientational molecular in-plane organization, where cholesterol molecules are located in the second coordination shell, avoiding direct cholesterol-cholesterol contacts, and forming a three-fold symmetric arrangement with proximal cholesterol molecules. At larger distances, the lateral three-fold organization is broken by thermal fluctuations. Other sterols having less structural asymmetry are found to lack the three-fold arrangement that is characteristic to cholesterol.

3483-Pos
Near-Field Structural Studies of Lipid Bilayers
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We use a Near-field Scanning Optical Microscope (NSOM) in conjunction with a Photo Elastic Modulator (PEM) to conduct birefringence (ne-n0) measurements with a spatial resolution of ~80nm. With our current setup we are able to distinguish changes in retardance S on the order of 10^-4 radians. Simultaneously while gathering information about S we extract information about the samples optical orientation φ referenced to the system’s axis, with an accuracy of ~7.24x10^-4 radians. We use our system on 1,2-dipalmitoylphosphatidylcholine (DPPC) bilayers, which at room temperature are in the gel state, (i.e.; their acyl chains have a ~320 degree azimuthal tilt with respect to the membranes normal). Modeling the membrane as a uniaxial crystal we are able determine the position of the acyl chains by measuring the birefringence and optical orientation. By controlling the temperature of our sample we hope to better study the structural changes that occur during phase transitions from gel to liquid states. The investigation of other lipid mixtures and the transformations they undergo during different phases will also be discussed.

3484-Pos
Calgary Lipids: A Lipid Force Field for Molecular Simulations
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Lipid bilayers are fundamental components of biological membranes. The most biologically relevant state of lipid bilayers is the fully hydrated fluid phase (fluid-disordered or liquid-ordered). Due to their fluidity, it is not possible to obtain experimentally atomic level structures of single bilayers. Computer simulations have been used to provide information about the structure of lipid bilayers, providing structural information that can aid the interpretation of experimental results. A large body of literature is available on simulations of lipid bilayers, but different studies used different simulation conditions and different force fields, which makes it difficult to compare results for different lipids. We present here a new united-atoms force field for lipids, compatible with common protein force fields. Partial charges and dihedral angles were derived from quantum mechanics calculations on lipid fragments, while Lennard-Jones parameters were tuned by fitting thermodynamic data for simple molecular building blocks. We built a library of structures of 60 different lipid bilayers, generated using molecular dynamics simulations in identical conditions. The library provides comparable structural data for bilayers with 4 different head groups and 15 pairs of acyl chains, including the most common lipid molecules. Structural parameters (such as the area per lipid and the electron density profiles) as well as dynamic properties (diffusion coefficients, deuterium order parameters) have been calculated for each bilayer, allowing for a systematic analysis of the effect of chain length, chain saturation and the chemical nature of the head group.

3485-Pos
Viscoelastic Properties of Plasma Membranes Varies with Cholesterol Level
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Cholesterol is an important lipid component of mammalian cell plasma membranes. It contributes to the biophysical properties of the plasma membrane, and plays an important role in the regulation of the membrane protein function.